EFFECT OF ADENOSINE AND INOSINE ON CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE IN RATS

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Abstract: Liver damage induced in rats by carbon tetrachloride (CCl₄) was obvious macroscopically as well as microscopically in stained sections. Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (γ-GT) were also significantly raised. Adenosine and inosine effectively countered the damage when these were given before and during the period during which CCl₄ produces the typical damage. The beneficial effect was seen in biochemical as well as pathological studies.

Key words: adenosine liver necrosis inosine carbon tetrachloride gamma glutamyl transpeptidase

INTRODUCTION

Carbon tetrachloride (CCl₄) induces fatty liver and liver cell necrosis (1). Though the precise mechanism is not known, several effects of CCl₄ seem to play a role such as inhibition of triacylglycerol release from the liver (1) and increase lipoperoxidation in membranes, whose structural integrity is necessary for lipoprotein release, finally resulting in liver triacylglycerol accumulation and destruction of liver cells. Adenosine is a component of the ubiquitous intracellular energy store, ATP, and the intracellular second messenger cyclic AMP. Its presence is associated with inhibition or stimulation of hormones, coronary vasodilation, delayed neurotransmission and changes in the metabolism of fat (2). Adenosine increases the cyclic AMP concentration in lymphocytes, brain slices, vagus nerve, platelets and myocardium while decreases cyclic AMP in fat cells, kidney cortex and liver (2). Adenosine released by the liver appears to play a significant role in replenishing erythrocyte ATP and so keeps the red cell alive (2), and prevent the induction of fatty liver by ethanol and cycloheximide (3). Frederiks (4) reported that adenosine - Mg Cl₂ reduced the extent of necrosis in rat liver subjected to ischemia. The present study was carried out in rats to explore if adenosine and inosine would counteract the biochemical and histological changes associated with CCl₄ induced liver damage in rats.

METHODS

Male albino rats of Wistar strain (HAU, Hissar, 120-140 g) with free access to standard diet (pellets, Hindustan Lever Limited, India) and tap water were used. Animals were divided into 5 groups of 10 each. Group I served as control (which received 3 injections of olive oil, 2 ml/kg, ip as vehicle). In Group II fresh mixture of CCl₄ + pure olive oil (1 : 1) was given on the 1st, 4th and 7th day in doses of 2 ml/kg, ip. The group II animals were also administered saline ip (vehicle for nucleosides) for 10 consecutive days. In groups III, IV and V, adenosine (100 mg/kg), inosine (100 mg/kg) and a prepared solution of hydrocortisone sodium succinate (10 mg/kg) respectively, were administered ip in addition to CCl₄. Adenosine, inosine and hydrocortisone solutions were given once daily, beginning one day prior to the experiments and continued for 10 consecutive days. On 10th day blood was withdrawn directly from the heart without using anesthesia and serum was separated by centrifugation for biochemical studies.

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Whole livers were removed after sacrificing the animals and preserved in 10% Formal saline. By the standard technique serial sections (5 μm) were cut and stained with haematoxyline and eosine. Aspartate aminotransferase, AST (EC 2.6.1.1) and alanine aminotransferase, (ALT; EC 2.6.1.2) levels in the serum of different groups were assayed (5). Gamma glutamyl transpeptidase (γ-GT : EC 2.3.2.2.) activity in serum was determined (6).

RESULTS

Liver from control group showed normal appearance, red colour, smooth and regular under surface without any evidence of haemorrhage and necrosis, while CCl₄ treated livers showed multiple area of necrosis with massive haemorrhagic patches. Most of livers were covered with white slough and there were multiple white patches indicating necrotic areas. All livers showed characteristic nutmeg appearance. The under surfaces of most of the livers were irregular/nodular. Livers from adenosine and inosine treated groups were almost normal in appearance except for mild congestion and minimum fatty changes. Livers from inosine treated group showed less congested areas as compared to adenosine treated group. Under surface of most of the livers from adenosine treated group were nodular, while undersurface of liver was smooth in inosine treated group. Hydrocortisone treated livers were normal in appearance regarding weight, colour and under surface.

Histology of liver from control group showed portal triad, rows of hepatocytes or normal arrangements of hepatocytes with nuclei, while CCl₄ treated liver sections showed intense centrilobular necrosis, sinusoidal haemorrhagic congestion and extensive fatty changes. Hepatocytes in centrilobular zone were enlarged and contained lipids. Hepatocytes in periportal zone were also enlarged and the normal architectural pattern was destroyed with severe vacuolization of surviving periportal hepatocytes (Fig.1). Histology of liver sections of adenosine + CCl₄ treated rats revealed few areas of congestion, spotty necrosis with minimum fatty changes (Fig. 2).

In inosine + CCl₄ treated group congestion was much less while fatty changes were more than the adenosine + CCl₄ - treated group. In contrast to adenosine + CCl₄ treated group there was no patches of necrosis in any liver sections from inosine + CCl₄ treated group.

Fig. 1 : Liver Section of CCl₄ treated rat showing intense centrilobular necrosis, haemorrhage (T) and extensive fatty change (low power : 10 x 10).

Fig. 2 : Liver Section from adenosine + CCl₄ treated rat showing spotty necrosis with minimum fatty change (10x10).
Hydrocortisone + CCl₄ - treated rat livers showed almost normal appearance except for mild fatty changes (Fig. 4).

Administration of CCl₄ to rats produced a marked elevation of serum AST, ALT and γ-GT levels (Table I). There was a marked reduction in serum AST, ALT and γ-GT levels in rats treated with adenosine + CCl₄, inosine + CCl₄ and hydrocortisone + CCl₄ as compared to animals treated CCl₄ alone.

**Table I**: Effect of adenosine and inosine on serum aminotransferase and gamma glutamyl transferase (γ-GT) in rats with CCl₄ induced liver damage. Values are mean ± SEM of 10 animals in each group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver Weight (% Body Weight)</th>
<th>AST IU/UL</th>
<th>ALT IU/UL</th>
<th>γ-GT IU/UL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.23±0.02</td>
<td>19.2±0.64</td>
<td>16.0±0.39</td>
<td>34.04±1.63</td>
</tr>
<tr>
<td>CCl₄</td>
<td>3.75±0.06</td>
<td>52.0±1.46</td>
<td>57.2±2.25</td>
<td>66.91±0.82</td>
</tr>
<tr>
<td>Adenosine + CCl₄</td>
<td>4.82±0.08*</td>
<td>24.1±0.32*</td>
<td>28.0±0.51*</td>
<td>45.51±0.31*</td>
</tr>
<tr>
<td>Inosine + CCl₄</td>
<td>5.10±0.05*</td>
<td>22.8±0.41*</td>
<td>26.2±0.61*</td>
<td>43.20±0.82*</td>
</tr>
<tr>
<td>Hydrocortisone+ CCl₄</td>
<td>4.34±0.03*</td>
<td>20.9±0.28*</td>
<td>23.8±0.46*</td>
<td>39.01±0.41*</td>
</tr>
</tbody>
</table>

*P<0.01 when compared with CCl₄ treated group (Unpaired 't' test)

**DISCUSSION**

Present study revealed marked protective effect of adenosine and inosine in carbon tetrachloride (CCl₄) induced liver damage in rats. The protective effect of adenosine and inosine may be related to their biochemical and metabolic actions like modulation of extracellular and intracellular Ca²⁺ concentration, providing precursor for ATP synthesis, antilipolytic, antiadrenergic, gluconeogenic, hypothermic and general vasodilator effects. Adenosine is reported to inhibit Ca²⁺ influx into myocardium, vascular smooth muscles and in various parts of brain (7, 8). It is possible that adenosine inhibits Ca²⁺ influx and modulates intracellular calcium which helps in preventing Ca²⁺ accumulation in liver cells, since it was demonstrated that cytosolic Ca²⁺ is elevated 100 folds in rat hepatocytes exposed to CCl₄ which is capable of initiating irreversible liver cell injury (9). It is well known that during myocardial ischemia/infarction break-down products of ATP such as ADP, AMP and adenosine may be utilized for the synthesis of ATP (10, 11). It is possible that adenosine and inosine increase the ATP production by providing precursor for the synthesis of ATP. Adenosine could diminish the break down of ATP by inhibiting of...
the deamination and dephosphorylation of ATP (12). The presence of high concentration of adenosine and inosine can probably act as buffer against diffusion thereby reducing adenine nucleotide degradation (10). Therefore, augmented ATP production and reduced ATP degradation following exogenously administered adenosine and inosine may be responsible for preventing CCl₄ induced liver damage in rats. Since ischemia and ATP depletion are prominent features in CCl₄ poisoning (1).

Well known antilipolytic effect of adenosine and inosine may be beneficial in preventing CCl₄ induced liver damage.

Recknagel (1) proposed that both hepatic necrosis and hepatic lipid accumulation in CCl₄ poisoning were due not to a direct effect of CCl₄ on liver cells but to a massive and persistent discharge of sympathetic nervous system by the action of CCl₄. Adenosine and other nucleotides reduced the release of almost all neurotransmitters whether they are inhibitory or excitatory and antagonize effects of catecholamines in various in vitro and in vivo preparations (13, 14). Hence, antiadrenergic effect may be beneficial in preventing CCl₄ toxicity.

REFERENCES